FORM PTO-1390
(REV. 11-94)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER
8484-010-999

08/637752

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP94/03564	October 28, 1994	October 28, 1993

TITLE OF INVENTION
ADENO-ASSOCIATED VIRUS - ITS DIAGNOSTIC USE WITH EARLY ABORTION

APPLICANT(S) FOR DO/EO/US
Andrea Kern, Jürgen Kleinschmidt, Karsten Geletneky, Michèle Rabreau, Jörg Schlehofer, Edda Tobiasch

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the international Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureaus.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 37(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☒ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:

a VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

02 MAY 1996
RECD PCT/PTO

EXPRESS MAIL CERTIFICATION

"Express Mail" label No. _____ Date of Deposit _____.
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Ofelia Rodriguez
(Type or print name of person mailing paper or fee)

(Signature of person mailing paper or fee)

INTERNATIONAL APPLICATION NO. PCT/EP94/03564	INTERNATIONAL FILING DATE 28 October 1994

17. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS				
(1)FOR	(2)NUMBER FILED	(3)NUMBER EXTRA	(4)RATE	(5)CALCULATIONS
TOTAL CLAIMS	25 -20=	5	X $ 22.00	$ 110
INDEPENDENT CLAIMS	5 -3=	2	X $ 78.00	156
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 250.00	250

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): **CHECK ONE BOX ONLY**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) . . . . . $ 680	
☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . . . . $ 750	
☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1010	1010
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) . . . . . $ 94	
☐ Filing with EPO or JPO search report . . . . . $ 880	
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than ☒ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).	130
TOTAL OF ABOVE CALCULATIONS	= 1656
Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (Note 37 CFR 1.9, 1.27, 1.28).	- 828
SUBTOTAL	= 828
Processing fee of $130.00 for furnishing the English Translation later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).	+
TOTAL FEES ENCLOSED	$ 828

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 16-1150 in the amount of $_____ to cover the above fees. A copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-1150. A copy of this sheet is enclosed.

18. ☐ Other instructions

19. ☐ All correspondence for this application should be mailed to

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NEW YORK 10036-2711

20. ☐ All telephone inquiries should be made to (212) 790-2803

ALBERT P. HALLUIN	Albert P. Halluin	25,227	4/29/96
NAME	SIGNATURE	REGISTRATION NUMBER	DATE

Express Mail No.: EL 340 684 876 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Kern *et al.*

Serial No.: 08/637,752 Group Art Unit: 1648

Filed: April 29, 1996 Examiner: M. Mosher

For: ADENO-ASSOCIATED VIRUS-ITS DIAGNOSTIC USE WITH EARLY ABORTION

Attorney Docket No.: 8484-013-999

AMENDMENT AND RESPONSE TO RESTRICTION REQUIREMENT

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Sir:

In response to the Office Action dated October 2, 2001 (Paper No. 14), please enter the following amendments and remarks.

Applicants enclose herewith a Petition for a one (1) month extension of time extending the time for response from November 2, 2001 to December 2, 2001.

AMENDMENTS

IN THE CLAIMS:

Please cancel Claims 1-11, and 14-15.

Please amend the claims as follows:

12. (Amended) A kit for detecting the causative agent of spontaneous abortion, comprising a probe antibody directed to an AAV antigen in a suitable container.

13. (Amended) The kit of Claim 12, wherein said probe antibody is A1 as deposited with DSM under deposit number ACC2195, A20 as deposited with DSM under deposit number ACC2194, A69 as deposited with DSM under deposit number ACC2196 or B1 as deposited with DSM under deposit number ACC2197.

16. (Amended) An antibody directed against an AAV antigen.

17. (Amended) The antibody of Claim 16, wherein said antibody is directed against an AAV capsid or a protein thereof.

18. (Amended) The antibody of Claim 17, wherein said antibody is A1 as deposited with DSM under deposit number ACC2195.

19. (Amended) The antibody of Claim 17, wherein said antibody is A20 as deposited with DSM under deposit number ACC2194.

20. (Amended) The antibody of Claim 17, wherein said antibody is A69 as deposited with DSM under deposit number ACC2196.

21. (Amended) The antibody of Claim 17, wherein said antibody is B1 as deposited with DSM under deposit number ACC2197.

REMARKS

Claims 1-11, 14, and 15 have been canceled as belonging to a non-elected invention. Claims 12, 13, and 16-21 are pending.

The Amendments. The claims have been amended to avoid dependencies from non-elected claims. The amendments do not introduce new matter, and they are fully supported in the specification and the claims as originally filed. Entry pursuant to 37 C.F.R. § 1.111 is therefore respectfully requested. A marked-up copy of the amended claims is attached hereto as *Appendix A*. The claims as presently pending are attached hereto as *Appendix B*.

Response to Restriction Requirement. In response to the requirement for restriction, Applicants hereby elect Group II, encompassing Claims 12, 13, 16-21, which are drawn to anti-AAV antibody products, without traverse. Applicants reserve the right to pursue Group I, encompassing Claims 1-11, and Group III, encompassing Claims 14-15, in later filed divisional applications.

No fee is believed to be due with this response. However, if it is determined that fees are due, please charge them to Pennie & Edmonds LLP Deposit Account No. 16-1150 (order no. 8484-013-999). A copy of this sheet is enclosed for accounting purposes.

Respectfully submitted,

Date 30 November 2001

Birgit Millauer 43,341 (Reg. No.)

for: Laura A. Coruzzi (Reg. No. 30,742)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
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APPENDIX A
MARKED-UP COPY OF THE AMENDED CLAIMS

IN THE CLAIMS:

Please cancel Claims 1-11, and 14-15.

Please amend the claims as follows:

12. (Amended) A kit for [performing the method according to Claim 4] detecting the causative agent of spontaneous abortion, comprising a probe antibody directed to an AAV antigen in a suitable container.

13. (Amended) The kit [according to] of Claim 12, wherein [the] said probe antibody is AI [(DSM] as deposited with DSM under deposit number ACC2195[, deposited on 13.10.1994)], A20 [(DSM] as deposited with DSM under deposit number ACC2194[, deposited an 13.10.1994)], A69 [(DSM] as deposited with DSM under deposit number ACC2196[, deposited on 13.10.1994 and/or] or Bl [(DSM] as deposited with DSM under deposit number ACC2197[, deposited on 13.10.1994)].

16. (Amended) [Antibody] An antibody directed [to] against an AAV antigen.

17. (Amended) [Antibody according to] The antibody of Claim 16, wherein [the] said antibody is directed [to] against an AAV capsid or a protein thereof.

18. (Amended) [Antibody according to] The antibody of Claim 17, wherein [the] said antibody is Al [(DSM] as deposited with DSM under deposit number ACC2195[, deposited on 13.10.1994)].

19. (Amended) [Antibody according to] The antibody of Claim 17, wherein [the] said antibody is A20 [(DSM] as deposited with DSM under deposit number ACC2194[, deposited on 13.10.1994)].

20. (Amended) [Antibody according to] The antibody of Claim 17, wherein [the] said antibody is A69 [(DSM] as deposited with DSM under deposit number ACC2196[, deposited on 13.10.1994)].

21. (Amended) [Antibody according to] The antibody of Claim 17, wherein [the] said antibody is Bl [(DSM] as deposited with DSM under deposit number ACC2197[, deposited on 13.10.1994)].

APPENDIX B

CLEAN COPY OF THE AMENDED CLAIMS

12. (Amended) A kit for detecting the causative agent of spontaneous abortion, comprising a probe antibody directed to an AAV antigen in a suitable container.

13. (Amended) The kit of Claim 12, wherein said probe antibody is A1 as deposited with DSM under deposit number ACC2195, A20 as deposited with DSM under deposit number ACC2194, A69 as deposited with DSM under deposit number ACC2196 or B1 as deposited with DSM under deposit number ACC2197.

16. (Amended) An antibody directed against an AAV antigen.

17. (Amended) The antibody of Claim 16, wherein said antibody is directed against an AAV capsid or a protein thereof.

18. (Amended) The antibody of Claim 17, wherein said antibody is Al as deposited with DSM under deposit number ACC2195.

19. (Amended) The antibody of Claim 17, wherein said antibody is A20 as deposited with DSM under deposit number ACC2194.

20. (Amended) The antibody of Claim 17, wherein said antibody is A69 as deposited with DSM under deposit number ACC2196.

21. (Amended) The antibody of Claim 17, wherein said antibody is Bl as deposited with DSM under deposit number ACC2197.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ADENO-ASSOCIATED VIRUS - ITS DIAGNOSTIC USE WITH EARLY ABORTION

This is a national phase filing of the Application No. PCT/EP94/03564, which was filed with the Patent Corporation Treaty on October 28, 1994, and is entitled to priority of European Patent Application 93117452.8, filed October 23, 1993.

I. FIELD OF THE INVENTION

The present invention relates to a method of detecting a causative agent of the so-called spontaneous early abortion by investigating patients' samples for the presence of adeno-associated virus DNA (AAV DNA), or AAV antigen or antibodies, preferably of the IgM type, directed to AAV. Furthermore, the present invention relates to antibodies suitable for said method.

II. BACKGROUND OF THE INVENTION

The adeno-associated viruses (AAV) which are human parvoviruses that depend on coinfecting helper viruses for their replication, are thought to be non-pathogenic (Siegl, et al. (1985), *Intervirology*, 23:61-73, Berns, *et al.* 1987, *Adv. Virus Res.* 32:243-306) but rather to exhibit tumorsuppressive properties (Rommelaere *et al.* 1991, J. Virol. Methods 33:233-251. The virus may persist in infected individuals, possibly by integration of its DNA into specific chromosomal sites of the host cell genome as seen in cell culture. Recent studies of our laboratories have demonstrated that AAV is able to induce differentiation in a variety of cells of human and mouse origin (Klein-Bauernschmitt *et al.* 1992, *J. Virol.* 66:4191-4200) including embryonic stem cells. In the course of looking for putative targets of AAV infection, we analyzed material from spontaneous abortion for the presence of AAV DNA using for

example the polymerase chain reaction (PCR), the Southern blotting technique and the in situ hybridization technique. Additionally, we analyzed serum samples from women with miscarriage and from other diseased or healthy women for the presence of antibodies to AAV using serological standard techniques such enzyme linked immunosorbent assay (ELISA), fluorescenceimmuno assay (FIA), radioimmune assay (RIA) or immunofluorescence assay (IFA).

Surprisingly, we found a significant correlation of both detectable AAV DNA in samples of abortion material and detectable IgM antibodies directed to AAV with the early abortion occurring during the first trimester of pregnancy.

III. SUMMARY OF THE INVENTION

The present invention relates to a method of detecting a causative agent of the so-called spontaneous early abortion by investigating patients' samples for the presence of adeno-associated virias DNA (AAV DNA), or AAV antigen or antibodies, preferably of the IgM type, directed to AAV. Furthermore, the present invention relates to antibodies suitable for said method.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts PCR analysis of DNA, prepared from histoligical sections of a spontaneous abortion (*see*, Examle 1).

V. BRIEF DESCRIPTION OF THE INVENTION

Accordingly, the present invention relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) hybridizing a probe for an AAV polynucleotide to nucleic acids of a sample of abortion material under conditions which allow the formation of a heteroduplex between an AAV nucleic acid and the probe, and

(b) detecting a polynucleotide duplex which contains the probe.

In a preferred embodiment of the present invention the method as mentioned above is a polymerase chain reaction (PCR), Southern blotting or in situ hybridization technique.

In another preferred embodiment of the present invention a hybridization technique is applied as described above, wherein one or more nucleic acid probes are used which are selected from the group consisting of the primers pan1, pan2, nest1 and nest2. In FIGURE 1 a schematic drawing of these primers, relative to the genome of the AAV type 2 (AAV-2) and the nucleotide sequences of the primers is presented.

The present invention further relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) incubating a probe antibody directed to an AAV antigen with a sample of abortion material under conditions which allow the formation of an antigen-antibody complex, and

(b) detecting the antigen-antibody complex containing the probe antibody.

In step (a) one or more probe antibodies can be used. These antibodies can be directed to e.g. an AAV capsid or a single protein thereof, particularly VP1, VP2 or VP3. Examples of these antibodies are the following monoclonals:
A1; deposited at DSM under DSM ACC2195 on Oct. 13, 1994
A69; deposited at DSM under DSM ACC2196 on Oct. 13, 1994
B1; deposited at DSM under DSM ACC2197 on Oct. 13, 1994
A20; deposited at DSM under DSM ACC2194 on Oct. 13, 1994
(*see*, TABLE 1).

The antibodies as mentioned above are subject matter of the present invention.

In a preferred embodiment of the present invention the method of antigen detection as mentioned above is an enzyme linked immunosorbent assay (ELISA), a radioimmuno assay (RIA), a fluorescence immuno assay (FIA) or an immunofluorescence assay (IFA).

An example of the ELISA comprises the following steps.

(a) providing a substrate carrying the monoclonal antibody A 20,

(b) contacting the substrate of (a) with a sample of abortion material to get an antigen-antibody complex,

(c) contacting the complex of (b) with a polyclonal anti-AAV capsid antibody to get an antibody-antigen-antibody complex,

(d) contacting the complex of (c) with an enzyme-labelled antibody directed to the polyclonal antibody of (c) to get a labelled complex of (c), and

(e) contacting the complex of (d) with an enzyme-label-indicator to indicate the presence of said complex.

It is clear that the term "sample of abortion material" is only an example of materials which contain AAV capsids or parts thereof. Other examples are cells expressing recombinant AAV capsids or parts thereof.

The present invention, *i.e.* the antibodies alone or in combination with the AAV antigen detection method, is suitable to detect AAV capsids and/or parts thereof in any material.

Furthermore, the present invention relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) incubating a sample containing AAV or an antigenic part thereof with a sample suspected of containing anti-AAV antibodies under conditions which allow the formation of an antibodyantigen complex, preferably only containing antibodies of the IgM type, and

(b) detecting an antibody-antigen complex, preferably Igm antibodyantigen complex, containing the probe antigen.

In step (a) the term "sample containing AAV or an antigenic part thereof" refers to AAV capsid proteins, particularly VPI, VP2 and/or VP3, preferably.

In another preferred embodiment of the present invention the method of detection of AAV specific antibodies, particularly IgM antibodies, is an ELISA, a RIA, a FIA or an IFA.

An example of the ELISA comprises the following steps:

(a) providing a substrate carrying an anti-human IgM

antibody,

(b) contacting the substrate of (a) with a patient's bodyfluid to got an antibody-antibody complex,

(c) contacting the complex of (b) with recombinant VP1, VP2 and/or VP3 to get a VP-antibody-antibody complex,

(d) contacting the complex of (c) with an anti-VP-antibody to get an anti-VP-antibody-VP-antibody-antibody complex,

(e) contacting the complex of (d) with an enzyme-labelled antibody directed to the anti-VP-antibody of (d) to get a labelled complex of (d), and

(f) contacting the complex of (e) with an enzyme-label-indicator to indicate the presence of said complex.

It is evident that persisting anti-AAV IgM/IgG titers in serum are associated with predisposition to early abortions. Thus, the present invention can also be used for effective risk factor screening, development of methods for prevention of pregnancy failure, and information of patients about the risks of pregnancy failure.

Furthermore, the present invention relates to a kit for detecting the causative agent of spontaneous abortion by hybridization as described above, comprising a probe for an AAV polynucleotide in a suitable container.

The present invention further relates to a kit for detecting the causative agent of spontaneous abortion by immunological antigen detection as described above, comprising a probe antibody directed against an AAV antigen in a suitable container.

The present invention further relates to a kit for detecting the causative agent of spontaneous abortion by immunological antibody detection as described above, comprising AAV or an antigenic part thereof in a suitable container.

Modes for carrying out the invention. The art is rich in methods available to the man of the art in recombinent nucleic acid technology, microbiology and immunobiology for carrying out the present invention. Detailed descriptions of all of these techniques will be found in the relevant

literature. See for example Maniatis, Fritsch & Sambrook: Molecular Cloning: A Laboratory Manual (1989); DNA Cloning, Vol. I and 11 (D.N. Glover ed., 1985); Oligonucleotide Synthesis (M.J. Gait ed., 1984); Nucleic Acid Hybridization (B.D. Hamos & S.J. Higgins eds., 1984); Animal Cell Culture (R.I. Freshney ed., 1986); J.D. Watson, M. Gilman, J. Witkowski, M. Zoller: Recombinant DNA, Second Edition (1992); Immunochemical Methods in Cell and Molecular Biology (Academic Press, London, 1387); Protein Purification: Principles and Practice, Second Edition (Springer Verlag, N.Y.); Handbook of Experimental lmmunology, Vol. I-IV (D.M. Weir and C.C. Blackwell eds., 1986); lmmunoassay: A Practical Guide (D.W. Chan and M.T. Perlstein eds., 1987). ELISA and Other Solid Phase lmmunoassays: Theoretical and Practical Aspects (D.M. Kemeny and S.J. Challacombe eds., 1988); Principles and Practice of lmmunoassay (C.P. Price and D.J. Newman eds., 1991).

More detailed information on specific methodological aspects of AAV, such as cell culture, virus growth, virus purification, isolation of proteins, can be found in the relevant literature, e.g. Handbook of Parvoviruses, Vol. I and 11 CRC Press, Boca Raton, Florida, Ed. P. Tijssen; Ruffing, *et al.* 1992, *J. Virol.*, 66:6922-6930.

All reagents such as antigens, antibodies, probe antigens, probe antibodies, nucleic acid probes, primers and auxilliary reagents necessary to perform an immunoassay or a hybridization assay, possibly using amplification techniques for improved sensitivity may be filled into suitable containers or coated to any solid phase such as plastic, glass and cells, and packaged into kits together with instructions for conducting the test.

VI. EXAMPLES

A. Example 1: Detection by Polymerase-Chain-Reaction (PCR) - analysis of AAV DNA in biological, e.g., curettage material of spontanious abortion.

The primers used in PCR (pan1, pan3) and nested PCR (nest1, nest2), respectively, were designed to hybridize to

sequences of AAV-2 and AAV-5 DNA by allowing mismatches not leading to amplification of other (e.g. cellular) DNA sequences. The amplified products are distinguishable by Southern blot experiments. The primers were prepared according to standard procedures.

The primers were designed displaying mismatches (underlined) as shown below:

```
      - - - - - - - AACTGGACCAATGAAAACTTTCC - - - - - - - -        pan1
1386  TGCGTAAACTGGACCAATGAGAACTTTCCCTTCAAC                        AAV-2
 130  TGCGTAAACTGGACCAATGAAAACTTTCCCTTCAAC                        AAV-5
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```
      AAAAAGTCTTTGACTTCCTGCTT                                     pan3
1729  AAAAAGTCTTTGACTTCCTGCTT                                     AAV-2
 472  AAAAAGTCCTTGACTTCCTGCTT                                     AAV-5
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DNA prepared from histological sections (5 μm, of fresh or fixed, paraffin-embedded, deparaffinated material (Methods as described by D.H. Wright and M.M. Manos in "PCR Protocols, A Guide to Methods and Applications", edited by M.A. Innis, D.H. Gelfand, J.J. Snoisky and T.L. White, Chapter 19, pp. 153-158; Academic Press, New York, 1990) were analysed by PCR using the primers pan1 and pan3 combined, followed in AAV posititive cases by a repetition of the PCR (to confirm specificity) using the (internal) primers nest1 and nest2 (*see*, FIGURE 1), respectively. PCRs were performed for 40 cycles (one cycle = 92°C, 1 min; 62 °C, 4 min; 92°C, 15 sec) (van den Brule *et al.*, (1989) *J. Med. Virol.*, 29:20-27). Amplified products were characterized by electrophoretic separation (2% agarose gel) and blotting onto a nylon membrane (Gene Screen, NEN, Dupont, Dreieich, Germany) followed by hybridization at high stringency with ^{32}P-labelled probes (labelled using the Megaprime™ DNA

Labelling System, Amersham, UK) of AAV-2 (pTAV2 [Heilbronn *et al.* (1990), J. Virol., 64, pp. 3012-3018) or of AAV-5. This probe was cloned from DNA from purified AAV-5 virions, propagated with adenovirus type 12 and purified as described in de La Maze and Carter (1980), J. Virol., 33. pp. 1129-1137 and in Rose (1974) Parvovirus Reproduction, pp. 1-61; In: H. Fraenkel-Conrat and R.R. Wagner, eds., Comprehensive Virology, Plenum Press, New York.

B. **Example 2: Detection by Southern Blotting analysis of AAV DNA in fresh curettage material.**

Genomic DNA was isolated using standard procedures with minor modification (Laird *et al.* 1991, *Nucl. Acids Res.*, 19:4293-4294) and digested with restriction enzymes allowing analysis of characteristic restriction sites within the AAV genome. After separation through 0,8 % agarose gels, DNA fragments were blotted onto Nylon membranes (Gene Screen) and hybridized AAV-2 DNA (pTAV2, *see*, Example 1) or specific AAV-5 DNA (*see*, TABLE 2) labelled by random priming with [α-^{32}P] dCTP (Amershem, Braunschweig, Germany).

C. Example 3: Detection of AAV DNA by in situ hybridization in sections of biopsy material, e.g. curettage from spontaneous abortion.

In situ hybridization was performed as described (Tobiasch *et al.* 1992, *Differentiation*, 50:163-178), however, with the modification that AAV-2 DNA was detected by RNA-DNA hybridization. After DNase treatment, the probes were subjected to limited alkaline hydrolysis. Upon linearisation of the plasmid pTAV2 (Heilbronn *et al.* 1990, *supra*, with EcoRV, riboprobes were obtained and labelled with [^{35}S]-UTP by in vitro transcription with T7 RNA polymerase (method as described in Boehringer Mannheim Procedure supplied with the "SP6/17 Transcription Kit"). Prior to hybridization, both probe and target DNA were denatured (93°C, 10 min). For in situ hybridization with [^{32}P-]-UTP labelled probes, the protocol was as described in Dürst *et al.* 1992, *Virology*,

189:132-140.

D. **Example 4: Provision Of Antibodies Directed To AAV Capsid Proteins**

In order to generate monoclonal antibodies directed to AAV capside proteins two BALB/C mice were injected subcutaneously (s.c.) with 150 µl of a mixture of gel purified recombinant capsid proteins in PBS containing 100 µg each of VP1, VP2 and VP3, mixed with an equal volume of complete Freund's adjuvant. After four weeks the mice were boosted s.c. with 25µg of purified UV-inactivated AAV-2 in 50 µl PBS and 50 µl incomplete Freund's adjuvant. After four weeks the mice were injected intraperitoneally (i.p.) each with 10 µg of UV-Inactivated AAV-2 in 100 µl PBS. Three days later one mouse was killed and the spleen cells were fused vvith X63/Ag8 cells according to standard procedures (Harlovv, E. and Lane, D. (1988), Cold Spring Harbor Laboratory, Antibodies, A laboratory mannual). Resultant hybridoma culture supernatants were screened by Western blotting, immunofluorescence and ELISA. The second mouse was immunized six months later with 100 µg of purified VP3 in PBS (i.p.) and monoclonal antibodies were prepared as described above.

E. **Example 5: ELISA For The Detection Of IgG Antibodies Directed To AAV**

96-well microtiterplates (Nunc, Denmark) were coated with 50 µl CsCl-gradient purified AAV 2 (dilution 1:1000 in 0,05 M carbonate-buffer pH 9,6) or with 50 µl recombinant AAV 2 capsid proteins VPl-3 (1:8000 in 0,05 M carbonate-buffer) and incubated overnight at RT. Plates were washed twice (washing buffer: PBS, 0,05 % Tween 20) and human sera were added (50 µl/well, dilutions 1:25 to 1:800, dilution buffer: PBS, 2% BSA, 0,05% Tween 20) and incubated for 1 h at 37°C in a wet chamber. After washing plates were incubated with 50 µl/well peroxydase conjugated monkey antihuman IgG antibody

(1:2000) for 45 minutes at 37°C in a wet chamber. Plates were washed four times and 50 µl substrate solution (5 mg OPD in 25 ml O,1 M citratebuffer pH 5,0 + 10 µl H_2O_2 35%) was added. Plates were stored for 10-15 minutes in the dark and the reaction was stopped with 50 µl 1N H_2SO_4/well. Extinctions were measured at 492 nm in a Titertek photometer. Background signal was determined by measuring the extinction without adding human sera and was substracted on every well (background signal extinction ranged from 0,035 to 0,05).

F. Example 6: ELISA For The Detection Of IgM Antibodies Directed To AAV

Version A

Plates were coated as described in Example 4. Human sera were added after they had been treated according to the following absorption protocol in order to eliminate remaining IgG-antibodies: 20 µl absorption reagent (FREKA-Fluor, Fresenius, Germany) were diluted with 25 µl PBS and 5 µl of human serum was added. Absorption was performed for at least 15 minutes at RT, and subsequently sera were tested at dilutions from 1:100 to 1:800. Incubation was performed for 1 h at 37°C in a wet chamber and after washing 50 µl/well peroxydase conjugated goat anti human IgM antibody (1:2000 in PBS/2 % BSA/0,05 % TWEEN 20) were added. Plates were incubated for 45 minutes at 37°C and washed four times. The OPD reaction and photometric evaluation were performed as described in Example 5.

Version B

µ-capture ELISA

Plate Coating

Rabbit anti-human IgM antibody (DAKO) was first denatured at a protein concentration of 600µg/ml, incubating for 30 min at RT in 50mM glycin/HCl pH 2,5 containing 100 mM NaCl then neutralized with 1 M Tris base. The denatured antibody was then desalted by passing the solution over a

Sephadex PD 10 column equilibrated in the coating solution (lOmM Tris/HCl pH 8,5 containing 100 mM NACl). The sample was eluted from the column in the same buffer. The solution was adjusted to a protein concentration of 6µg/ml by dilution in coating buffer and 200 µl added to each well on a polystyrene microtiter plate (NUNC immuno flat-bottomed well). The plate was incubated at 37°C for 24 h in a humid atmosphere, contents decanted and wells washed 4 times with 250 µl/well of Tris-buffered saline (TBS) (0,02 M Tris/HCl pH 7,4, 0,15 M NaCl) containing 0,05 % Tween 20 (wash buffer). The wells were then blocked with TBS containing 1% Tween 20 and 5 % Sucrose (blocking solution) by incubating at 4°C followed by 2 washings in wash buffer (TBS containing 0,05% Tween 20).

Assay

The second step in the ELISA involved contacting patients' sera with the antibody-coated plate. During incubation, IgM was immunologically bound to the solid-phase antibody. After removal of the unbound material and washing of the microtiter plates, the plates were incubated with purified recombinant AAV nucleocapsid proteins VP1, VP2 and VP3. After removal of the unbound material and washing of the microtiter plates, complexes of human IgM antibody-VP complexes were detected by incubation with the A1, A69 and B1 antibodies. Unbound monoclonal antibodies were removed by aspiration and the plates were washed. The bound monoclonal antibodies were detected by incubating the plates with goat anti-mouse immunoglobulin antibodies conjugated to horseradish peroxidase (HRP). Following removal of unbound conjugate by washing, a solution containing H_2O_2 3-3', 5-5' tetramethylbenzidine (TMB) was added. Reactions were stopped after a suitable interval by addition of sulfuric acid. The Cutoff value of the ELISA was calculated as the average optical density of five negative samples plus 3 standard deviations (to correct for any aspecific binding). Samples giving absorbance values higher then the cutoff were

considered positive.

Specifically, the anti-human IgM on the plate was reacted with serum by adding 100µl of serum samples diluted 1:200 in TBS containing 10 mg/ml bovine serum albumin, and incubating the serum-containing wells for 1 h at room temperature. After incubation, the serum samples were removed by aspiration and the wells were washed 5 times with washing solution (TBS + 0,05% Tween 20). Aliquots of 100µl of the VP1, VP2 and VP3 antigen mixture (conc of 10-10 nM VP1, VP2 and VP3) were added to each well and the plates were incubated at room temperature at least 2 h, followed by removal of excess probe by aspiration and 5 washes with TBS + 0,05 % Tween 20. Bound VP1, VP2 and VP3 was detected by addition of 100µl of a mixture of hybridoma supernatants from A1, A69 and B1 monoclonal antibodies producing hybridomas (antibody conc 1-10 nM), followed by 5 standard washes of the plates with TBS + 0,05% Tween 20. Monoclonal antibody binding was detected by addition of 200µl of 1 1/2000 dilution of sheep anti-mouse IgG horseradish peroxidase-conjugated antibody (Dako, Hamburg/Germany) and incubated for 1,5 h at room temperature, followed by 5 standard washings of the plate. Enzyme activity was revealed by addition of 100µl of a solution of TMB (Serex, Maywood, N.J./USA). The plate was incubated until the desired color development was reached and terminated by addition of 50,µl 2N sulfuric acid. Optical densities (OD_{450}) of negative and positive control sera as well as samples were determined. The cutoff value as calculated from five negative sera was OD_{450}=0,40.

G. Example 7: ELISA For The Detection Of AAV Capsids Plate Coating

100 µl of the A20 antibody (*see, supra*) equilibrated in coating buffer solution (50 mM $NAHCO_3$ pH 9.6 and adjusted to a protein concentration of 1,5 ng/ml was addad to each well on a polystyrene microtiter plate (NUNC immune flat-bottomed well). The plate was incubated at 4°C for 24 h,

contents decanted and wells, washed 5 times with 250 µl/well of phosphate-buffered saline (PBS) (wash buffer). The wells were blocked with 260 µl of 3% BSA in PBS (blocking solution) by incubating at least 30 minutes at room temperature followed by 6 washings in wash buffer.

Assay

A standard curve within the range of 10 - 10,000 capsids/ml was prepared by diluting AAV capsids in standard dilution solution containing PBS.

Unknown samples were diluted as appropriate in dilutent solution and 100 µl added to the test wells. When tissue culture supernatants were to be assayed, 100 µl of a 1:10 to $1:10^8$ dilution was to be added to the test well. The plate was incubated for 3 h at room temperature. The plate was washed 5 times in wash buffer and 100 µl rabbit anti-AAV-polyclonal antiserum at a dilution of 1/1000 in 3% BSA in PBS added to each well. The plate was incubated at room temperature for 2 h as previously and then washed 5 times in PBS Tween. AAV capsid was detected by addition of 100 µl of a 1/2000 dilution of a goat anti-rabbit IgG myeloperoxidase-conjugated antibody prepared in antibody diluent and incubated for 1 h at room temperature followed by 5 standards washes of the plate. Enzyme activity was revealed by addition of 100 µl of a 0.1 mg/ml solution of tetramethylbenzidine (TMB) prepared in O,1 M Na-acetate buffer pH 6 to each well. The plate was incubated at room temperature until the desired color development was reached, longer incubation periods being necessary to detect lower concentration ranges, *i.e.* standards less than 10 capsids/ml. The concentration of unknown samples was determined by comparison of their optical density to the standard curve.

H. Example 8: Detection of AAV-DNA In Curettage Materil Of Spontaneous Absorption

A total of 50 samples of curettage material of spontaneous absorption were analysed for the presence of AAV

DNA either by PCR or Southern Blotting or both. 41 samples were from abortions in the first and 9 samples from abortions in the second and third trimester of pregnancy.

Among the 41 samples taken during the first trimester of pregnancy, 14 consisted of fresh material that could be tested by Southern Blotting, by which method 9 samples were shown to be positive. All other samples tested were sections from paraffin-embedded tissues, that were analysed by PCR. Among these, 30 samples were from abortions in the first trimester of pregnancy, of which 12 samples were shown to be positive for AAV DNA. All of the 9 samples from the second or third trimester of pregnancy were negative by PCR.

Thus, in 21 of 41 samples, *i.e.* 50% of spontaneous abortions in the first trimester of pregnancy AAV specific DNA sequences could be detected, whereas 9 spontaneous abortions in the second or third trimester were negative (see TABLE 3).

I. Example 9

A total serum of 148 serum samples drawn from healthy probands, diseased patients with various syndromes being unrelated to abortion, and pregnant women with spontaneous abortion during the first trimester of pregnancy were tested for antibodies directed to AAV.

The results obtained are displayed in TABLE 4. Generally, the prevalence of specific IgG antibodies was quite high, between 62 and 100% in the different groups of probands/patients. However, specific IgM antibodies were shown to be significantly correlated with "pregnancy problems".

Table 1

Term	Subtype	Epitope	Western Blotting	Immuno-Precipitation	Immuno-Fluorescence	Characteristics
A1	IgG2a	between aa 1-104	+ specific recognition of VP1	+	+	recognition of monomeric and oligomeric VP1
A69	IgG1	between aa 105-136	+ specific recognition of VP1 and VP2	++	++	recognition of monomeric and oligomeric VP1 and VP2
B1	IgG1	between aa 136-669	+ + recognition of VP1, VP2 and VP3	++	++	recognition of monomeric and oligomeric VP1, VP2 and VP3
A20	IgG3	presumable conforma-tion	- (negativ)	+++	++	preferable recognition of AAV capsid, no reaction with recombinant monomeric capsid protein

aa: amino acid6

TABLE 2

388 bp part of BamH1b fragment of AAV5

```
                TCAATCAGGTGCCGGTGACTCACGACTTTAAAGTTCCCAGGGAATTGGCGGGAACTAAAG
487             AGTTAGTCCACGGCCACTGAGTGCTCAAATTTCAAGCCTCCCTTAACCGCCCTTGATTTC   546

                GGGCGGAGAAATCTCTAAAACGCCCACTGGGTGACGTCACCAATACTAGCTATAAAAGTC
547             CCCGCCTCTTTAGAGATTTTGCGGGTGACCCACTGCAGTGGTTATGATCGATATTTTCAG   606

                TGGAGAAGCGGGCCAGGCTCTCATTTGTTCCCGACACGCCTCGCAGTTCAGACGTGACTG
607             ACCTCTTCGCCCGGTCCGAGAGTAAACAAGGGCTCTGCGGAGCGTCAAGTCTGCACTGAC   666

                TTGATCCCGCTCCTCTGCGACCGCTCAATTGGAATTCAAGGTATGATTGCAAATGTGACT
667             AACTAGGGCGAGGAGACGCTGGCGAGTTAACCTTAAGTTCCATACTAACGTTTACACTGA   726

                ATCATGCTCAATTTGACAACATTTCTAACAAATGTGATGAATGTGAATATTTGAATCGGG
727             TAGTACGAGTTAAACTGTTGTAAAGATTGTTTACACTACTTACACTTATAAACTTAGCCC   786

                GCAAAAATGGATGTATCTGTCACAATGTAACTCACTGTCAAATTTGTCATGGGATTCCCC
787             CGTTTTTACCTACATAGACAGTGTTACATTGAGTGACAGTTTAAACAGTACCCTAAGGGG   846

                CCTGGGAAAAGGAAAACTTGTCAGATTT
847             GGACCCTTTTCCTTTTGAACAGTCTAAA   874
```

Table 3

Prevalence of AAV DNA in curettage materials

	Detection of AAV DNA by (number AAV positive / number analysed)		
Diagnosis/Pathology	PCR	Southern Blotting	Total
spontaneous abortion (1st trimester of pregnancy)	12/30	9/14	21/41*
abortion 2nd trimester	0/3	n.d.	0/3
abortion 3rd trimester or placenta post partum	0/6	n.d	0/6

n.d. = not done;

* = 3 samples positive with PCR were tested by Southern blotting analysis

PEMP-54809.1

Table 4

Serum Antibodies to AAV Diagnosis	n	IgG- IgM-	IgG+ IgM-	IgG- IgM-	IgG+ IgM+	IgG+ n	%	IgM+ n	%
Controls (all)	58	8	45	2	3	48	83	5	8,6
Employees	32	4	24	2	2	26	81	4	12,5
Patents *)	26	4	21	0	1	22	85	1	4
breast (all)	38	1	32	0	5	37	97	5	13,2
mammary dystrophy	19	1	13	0	5	18	75	5	26
breast cancer	19	0	19	0	0	19	100	0	0
cervix uteri (all)	26	2	17	4	3	20	77	7	27
normal (or metaplasia)	3	1	2	0	0	2	67	0	0
CIN / CIS	22	1	14	4	3	17	77	7	32
cancer	1	0	1	0	0	1	100	0	0
pregnancy problems (all)	26	6	12	2	6	18	69	8	31
Extra uterine	2	0	2	0	0	2	100	0	0
chromosomal aberrations	3	0	2	0	1	3	100	1	33
abortion (1st trimester) of unclear etiology	21	6	8	2	5	13	62	8	38

*) with uterus myoma, or normal pregnancy, hysterectomy (normal)

CLAIMS:

1. A method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) hybridizing a probe for an AAV polynucleotide to nucleic acids of a sample of abortion material under conditions which allow the formation of a heteroduplex between an AAV nucleic acid and the probe, and

(b) detecting a polynucleotide duplex which contains the probe.

2. The method according to Claim 1, which is a PCR, Southern blotting or an in situ hybridization technique.

3. The method according to Claim 1, wherein one or more probes are used which are selected from the group consisting of the primers pan1, pan3, nest1 and nest2.

4. A method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) incubating a probe antibody directed to an AAV antigen with a sample of abortion material under conditions which allow the formation of an antigen-antibody complex, and

(b) detecting the antigen-antibody complex containing the probe antibody.

5. The method according to Claim 4, wherein the probe antibody is A1 (DSMACC2195, deposited on 13. 10.1994), A20 (DSM ACC2194, deposited on 13. 10. 1994), A69 (DSM ACC2196, deposited on 13. 10. 1994) and/or Bl (DSM ACC2197, deposited on 13. 10. 1994).

6. Tho method according to Claim 4 or 5, which is an ELISA, a RIA, a FIA or an IFA.

7. A method of detecting the causative agent of spontaneous abortion comprising the steps of

(a) incubating a sample containing AAV or an antigenic part thereof with a sample suspected of containing anti-AAV antibodies under conditions which allow the formation of an antibody-antigen complex, and

(b) detecting the antibody-antigen complex, containing the probe antigen.

8. The method according to Claim 7, wherein the antigenic part of AAV is VP1, VP2 or VP3.

9. The method according to Claim 7 or 8, wherein the antibody in the antibody-antigen complex is of the IgM type.

10. The method according to one of Claim 7 to 9, which is an ELISA, a RIA, a FIA or an IFA.

11. A kit for performing the method according to Claim 1, comprising a probe for an AAV polynucleotide in a suitable container.

12. A kit for performing the method according to Claim 4, comprising a probe antibody directed to an AAV antigen in a suitable container.

13. The kit according to Claim 12, wherein the probe antibody is AI (DSM ACC2195, deposited on 13.10.1994), A20 (DSM ACC2194, deposited an 13.10.1994), A69 (DSM ACC2196, deposited on 13.10.1994 and/or B1 (DSM ACC2197, deposited on 13.10.1994).

14. A kit for performing the method according to Claim 7, comprising AAV or an antigenic part thereof in a suitable container.

15. The kit according to Claim 14, wherein the antigenic part of AAV is VP1, VP2 and/or VP3.

16. Antibody directed to an AAV antigen.

17. Antibody according to Claim 16, wherein the antibody is directed to an AAV capsid or a protein thereof.

18. Antibody according to Claim 17, wherein the antibody is A1 (DSM ACC2195, deposited on 13.10.1994).

19. Antibody according to Claim 17, wherein the antibody is A20 (DSM ACC2194, deposited on 13.10.1994).

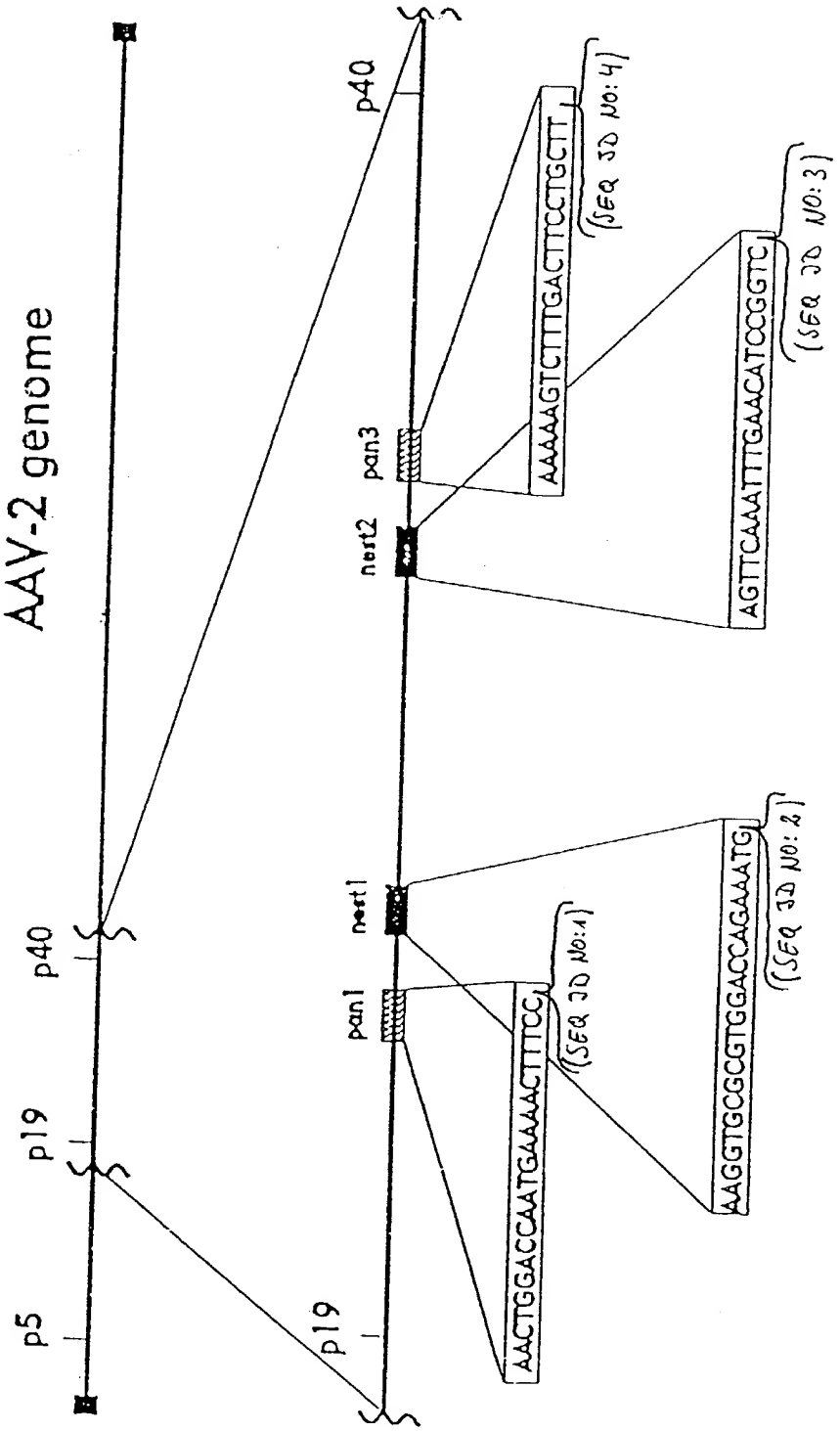
20. Antibody according to Claim 17, wherein the antibody is A69 (DSM ACC2196, deposited on 13.10.1994).

21. Antibody according to Claim 17, wherein the antibody is B1 (DSM ACC2197, deposited on 13.10.1994).

ABSTRACT

Adeno-Associated Virus-
Its Diagnostic Use with Early Abortion

The present invention relates to a method of detecting a causative agent of the so-called spontaneous early abortion by investigating patients' samples for the presence of adeno-associated virias DNA (AAV DNA), or AAV antigen or antibodies, preferably of the IgM type, directed to AAV. Furthermore, the present invention relates to antibodies suitable for said method.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(SEQ ID NO:2)
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(SEQ ID NO:3)
AAAAAGTCTTTGACTTCCTGCTT
(SEQ ID NO:4)

Figur 1

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As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

ADENO-ASSOCIATED VIRUS - ITS DIAGNOSTIC USE WITH EARLY ABORTION

and for which a patent application:
☐ is attached hereto and includes amendment(s) filed on _____ *(if applicable)*
☒ was filed in the United States on _____ as Application Serial No. 08/637,752 *(for declaration not accompanying application)*
with amendment(s) filed on _____ *(if applicable)*
☒ was filed as PCT international application Serial No. PCT/EP94/03564 on 28 October 1994 and was amended under PCT Article 19 on _____ *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
93117452.8	Europe	28 October 1993	YES ☒ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

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		PATENTED	PENDING	ABANDONED

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SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
RABEAU		
DATE 23/5/01	DATE 19/4/2001	DATE 14.06.2001

LIST OF REFERENCES CITED BY APPLICANT

(Use several sheets if necessary)

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APPLICANT	
Andrea Kern *et al.*	
FILING DATE	**GROUP**
April 29, 1996	N/A

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES	NO
MM	AA	WO 91/12269	8/22/91	PCT				
↓	AB	WO 91/04330	4/4/91	PCT				

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<210> 4

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer pan3

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<210> 5

<211> 36

<212> DNA

<213> Adeno-Associated Virus-2

<400> 5
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<210> 6

<211> 36

<212> DNA

<213> Adeno-Associated Virus-5

<400> 6
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<210> 7

<211> 23

<212> DNA

<213> Adeno-Associated Virus-2

<400> 7
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<210> 8

<211> 23

<212> DNA

<213> Adeno-Associated Virus-5

<220>

<223> Description of sequence: Sequence fragment from Adeno-Associated Virus-5

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<210> 9

<211> 388

<212> DNA

<213> Adeno-Associated Virus-5

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tggagaagcg ggccaggctc tcatttgttc ccgagacgcc tcgcagttca gacgtgactg 180

ttgatcccgc tcctctgcga ccgctcaatt ggaattcaag gtatgattgc aaatgtgact 240

atcatgctca atttgacaac atttctaaca aatgtgatga atgtgaatat ttgaatcggg 300

gcaaaaatgg atgtatctgt cacaatgtaa ctcactgtca aatttgtcat gggattcccc 360

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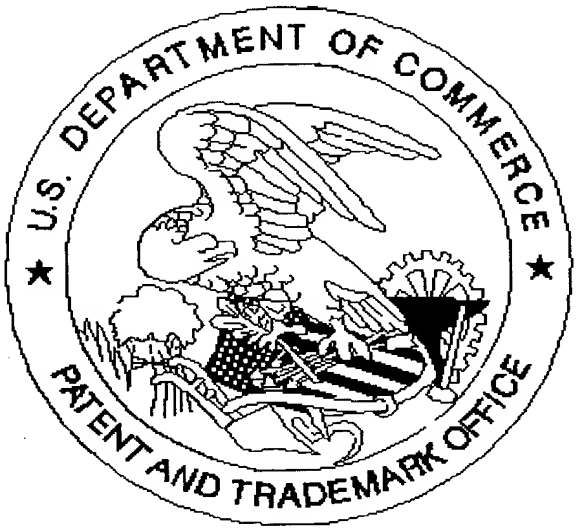

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